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Telomeres are repeated double stranded DNA sequences at chromosome ends with G rich 3'-ends (G-strand) and C rich 5'-ends (C-strand). Telomerase is the telomere G-strand synthesis enzyme. Recent data suggested that telomere replication might be involved in the origin or progression of cancer and aging. Understanding telomere replication might offer ways for screening drugs against and preventing cancer and aging.

The conservation of DNA replication and telomere structure has made yeast a good system to study telomere replication. An essential telomere protein in budding yeast, Cdc13p, has been proposed to bind telomeres to limit C-strand degradation and to recruit telomerase to telomeres. These steps are necessary for telomere replication. To better understand telomere replication, a two-hybrid assay was used to identify proteins interacting with Cdc13p. We found Pol1p, the catalytic subunit of DNA polymerase α (pol α), and Est1p, a telomerase component, interacted with Cdc13p. These interactions were confirmed by biochemical criteria. Disruption of the Pol1p-Cdc13p interaction resulted in longer telomeres. Data suggested that the pol α is the C-strand resynthesis enzyme. The balance of the opposite movements of telomerase and Pol α during telomere replication is one of the mechanisms to control telomere length. Cdc13p is a key protein to control this balance through interaction with Est1p and Pol1p. These data should help us to further understand mammalian telomere replication.

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Table of Contents

-INTRODUCTION

Telomeres are repeated double stranded DNA sequences at chromosome ends with G rich 3'-ends (G-strand) and C rich 5'-ends (C-strand). Telomerase is the telomere G-strand synthesis enzyme. Recent data suggested that telomere replication might be involved in the origin or progression of cancer and aging. Understanding telomere replication might offer ways for screening drugs against and preventing cancer and aging.

The conservation of DNA replication and telomere structure has made yeast a good system to study telomere replication. An essential telomere protein in budding yeast, Cdc13p, has been proposed to bind telomeres to limit C-strand degradation and to recruit telomerase to telomeres. These steps are necessary for telomere replication. Cdc13p might be a key regulatory protein in telomere synthesis.

BODY:

The purpose of the project is to further study telomere replication and its regulation using budding yeast as a model system. Since Cdc13p might be an important regulatory protein in yeast telomere replication, it might be expected to interact with numbers of telomere proteins such as: telomerase components, telomere replication proteins or/and their regulatory proteins, telomere structure proteins or/and proteins that regulate Cdc13p activities. Identifying these proteins and studying their interactions and the importance of the interactions might give us more knowledge about telomeres and telomere replication. Dr. Virginia A. Zakian is a pioneer in yeast telomere studies. Her lab not only discovered the Telomere Position Effect on transcription of nearby genes and on recombination of nearby DNA sequences, also identified the C-strand degradation as a necessary step before telomerase action. Princeton University has an excellent science research environment. Study yeast telomere replication in her lab in Princeton University will benefit my future.

To further study telomere replication, we planned a two-hybrid assay to screen and identify proteins interacting with Cdc13p. An N-terminal fragment of Pol1p, the catalytic subunit of DNA polymerase α (pol α), was isolated form the library screen. The interaction was confirmed by a immunoprecipitation using endogenously expressed proteins. In order to understand the importance of the interaction, Pol1p mutations that disrupt or reduce the interaction with Cdc13p in the two-hybrid system were generated by random PCR mutagenesis. In this period time supported by DAMA17-98-1-8145, I isolated three mutations that disrupt the two-hybrid interaction and had normal protein expression. They clustered in a very small region of Pol1p: D236N, E238K and P241T. The mutations were then integrated into the *POL1* locus so that the only copy of Pol1p is the mutated form. Strains that express the mutated pol1p had elongated telomeres but showed no noticeable growth defect and abnormal telomere structure such as long G-tails. The degree of the elongation of telomeres was correlated to the extent of the loss of

the Cdc13p-Pol1p two hybrid interaction. These data indicated that the Cdc13p-Pol1p interaction is specific for telomere replication, not involved in overall DNA replication.

To confirm the longer telomere phenotype in the Pol1p is due to the loss or reducing of Cdc13-Pol1p interaction, two-hybrid mutations in Cdc13p were also generated by PCR mediated mutagenesis. Numbers of mutations were identified to loss or reducing the two-hybrid interaction with Pol1p. They spread over Cdc13p. Two of the mutations were integrated into the *CDC13* locus on chromosomes. Consistently, strains carried the mutation alleles of *cdc13* also showed the same phenotype as *pol1* mutations: longer telomeres.

We also found that a C-terminal fragment of Est1p, protein required for telomerase activity *in vivo*, interacts with Cdc13p in the two hybrid assay. In this period time supported by DAMD17-98-1-8145, I confirmed the interaction by a biochemical assay, GST-pull-down assay, using overexpressed proteins. Since Est1p is not the telomerase catalytic subunit, and it might be function as a recruiter of telomerase, I proposed that Cdc13p recruits telomerase complex to telomeres by interacting with Est1p for the G-strand synthesis. I also proposed that the Pol α is the C-strand re-synthesis enzyme. The direction of the G-strand and the C-strand synthesis is opposite. Cdc13p balances the two opposite movements by interacting with Pol1p and Est1p. The balance of the two opposite movements is one of the mechanisms to regulate telomere length. These data should help us to further understand mammalian telomere replication.

APPENDED TO THE SUMMARY

1. Research Accomplishments:

   -Identified the interaction between Cdc13p and Pol1p, the catalytic subunit of DNA polymerase α.
   -Identified interaction between Cdc13p and Est1p, an essential protein for telomerase activity.
   -Discovered the importance of the Cdc13p-Pol1p interaction.
   -Discovered that Pol1p is responsible fore the C-strand synthesis during telomere replication.
   -Discovered that a new role of Cdc13p in telomere replication is to control telomere length.

2. Reportable Outcome

   Manuscript in preparation:

The Saccharomyces telomere binding protein Cdc13p interacts with both the catalytic subunit of DNA polymerase I and the telomerase associated Est1p protein